IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	: 10/530,094	Confirmation No.	7464
Applicant	: Audun Tornes		
Filed	: January 26, 2006		
Title	: METHOD, COMPOUNDS AND PREPARATIONS FOR THE IDENTIFICATION OF SENTINEL LYMPH NODES		
TC/Art Unit	: 1618		
Examiner:	: J.R. Samala		
Docket No.	: PN0275		
Customer No.	: **36335**		

Commissioner for Patents
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DECLARATION OF PER C. SONTUM PH.D. UNDER 37 C.F.R. § 1.132

I, Per C. Sontum, do hereby declare that:

1. I have been a Principal Scientist at GE Healthcare since 2004. Before that, I was a Senior Research Scientist at Amersham Health Plc. from 1997 to 2004, before it became GE Healthcare in 2004. I was a Research Scientist at Nycomed A/S from 1989 to 1994 and a Senior Research Scientist from 1994 to 1997 before Nycomed became Amersham in 1997. Prior to Nycomed, I was a Product Manager at Invicta A/S from 1987 to 1988.

2. I received my Ph.D. in Galenic Pharmacy in 2001 and my Masters of Science degree in Polymer Chemistry from the University of Oslo in 2001 and 1986, respectively. I received my Engineering Degree in Polymer Technology from the Oslo College of Engineering in 1982.

3. Much of the research I have performed at GE Healthcare (previously Nycomed and Amersham) from 1989 to date has focused on the physicochemical characterization of contrast agents for ultrasonography using gas filled microspheres. Over the years this work expanded to include other disperse formulations such as solid particles, liposomes, colloidal iron oxide, macromolecular systems, dendrimers, and the like.

4. My responsibilities as a research scientist at GE Healthcare, Amersham, and Nycomed included evaluation and procurement of instruments, instrument qualification, development and validation of analytical methods, research and GxP related analyses, reporting and evaluation of results, and the like. The main techniques applied were Coulter counting, laser diffraction, photon correlation spectroscopy, laser Doppler velocimetry, densitometry, sonometry, and automated image analysis. At peak workload my research group consisted of 3-4 engineers and 2 research scientists. Based on the work performed by this group, I wrote my dissertation for the Ph.D. entitled "Physicochemical Characterization and Characteristics of Disperse Pharmaceutical Formulations for contrast enhancement in Medical Imaging" in 2001.

5. I have reviewed the claims that are currently before the examiner in the captioned application. I understand that the invention is generally directed to a method for the identification of a sentinel lymph node in a subject by first administering microbubbles comprising at least 50% negatively charged phospholipids; allowing the microbubbles to accumulate in the sentinel lymph node; and detecting the microbubbles in the sentinel lymph node using ultrasound.

6. I have reviewed and analyzed the final Office Action dated December 17, 2010 ("the Office Action"), which issued in connection with the captioned application. In the Office Action, claims 1, 2, 6, and 7 were rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Published PCT Appl. No. WO00/45855 to Mattrey *et al.* ("Mattrey") in view of U.S. Patent No. 6,221,337 to Dugstad *et al.*

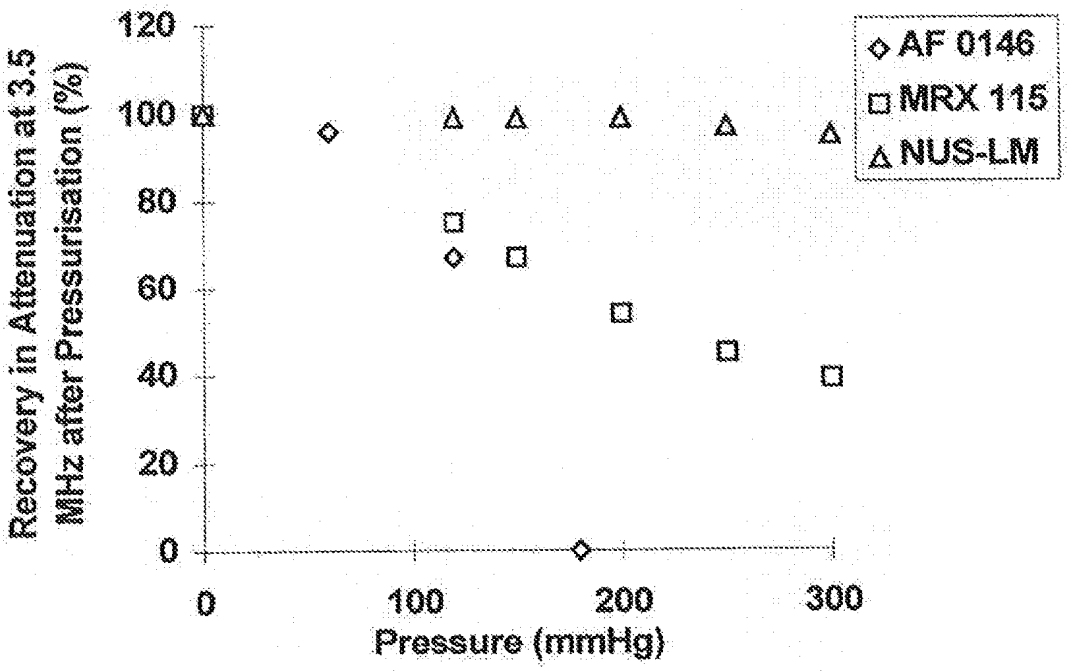
7. Briefly, Mattrey provides a general disclosure regarding the use of "microbubbles" for identifying the sentinel lymph node. Mattrey's microbbubles are purportedly made from a myriad of materials including lipids (i.e., phospholipids). In his disclosure, Mattrey references specific products, including, MRX115, and Imagent®, both of which comprise phospholipids. The shell of the MRX115 microbubble comprises phospholipids and polyethyleneglycol-phospholipids bearing an overall neutral charge and the shell of

Imagent® comprises phospholipids bearing an overall neutral charge. But Mattrey does not appear to disclose any phospholipid-containing products bearing an overall negative charge.

8. In 1995, I personally conducted stability studies comparing the pressure stabilities of the products Sonazoid®, MRX115, and Imagent®. Unlike MRX115 and Imagent®, Sonazoid® comprises phospholipids bearing an overall negative charge. Accordingly, Sonazoid® represents at least one embodiment encompassed by the pending claims.

9. The data from the study comparing the pressure stabilities of each product showed that Sonazoid® retained more than 95% of its initial attenuation efficacy after pressurization at a pressure of up to 300 mm Hg and was thus significantly more pressure stable than both MRX 115 and Imagent®. Sonazoid® would therefore be expected to be highly stable during administration and during the ultrasound examination.

10. More specifically, the data, shown below, show that MRX115 and Imagent® (referred to as "AF0146" in the graph) do not recover 100% of their attenuation at 3.5 MHz after exposure to a pressure of about 120 mm Hg. The recovery in attenuation of MRX115 and Imagent® decreases by over 20% after exposure to that pressure. The recovery in attenuation of both products is impacted drastically as the pressure is increased beyond 120 mm Hg. For example, the recovery in attenuation of Imagent® does not recover **at all** when the product is exposed to about 180 mm Hg. The recovery in attenuation of MRX115 appears to decrease linearly as a function of pressure. For example, at about 190 mm Hg, MRX115 recovers less than 60% of its attenuation. In contrast, Sonazoid® (referred to as "NUS-LM" in the graph) recovers 100% of its attenuation at 3.5 MHz after exposure to 120 mm Hg and even after exposure to 300 mm Hg.



11. The pressure stability reviewed in paragraph 10 is a predictor of the functionality of the various products for identification of sentinel lymph nodes. During subcutaneous injection hydrostatic pressures typically reach more than 300 mmHg. In this case significant amounts of the microbubbles in MRX115 and all of the microbubbles in Imagent® will be destroyed *during* injection. After injection the microbubbles are exposed to a variety of stress factors incurred by, *e.g.*, flow through micro vessels and variance in hydrostatic pressure. The pressure stability is a relevant predictor of the rate of microbubble destruction *in vivo*. Accordingly, Sonazoid® will display a superior persistency to that of MRX115 and Imagent® after administration.

12. The pressure stability is also a relevant predictor of microbubble destruction during imaging. Exposure to ultrasound is equivalent with exposure to applying pressure to the microbubbles. The relative stability demonstrated by the graph shown above translates to stability during exposure to ultrasound. Accordingly, Sonazoid® will allow for a significantly longer imaging window than MRX115 and Imagent®. This effect has been

demonstrated in experiments (data not shown here) where, at relevant acoustic pressures, MRX115 was shown to be completely destroyed after only about nine seconds of ultrasound exposure whereas Sonazoid® was only marginally affected after the same exposure.

13. The data presented above demonstrate that Sonazoid® is far more stable with regard to pressurization than MRX115 and Imagent®. From a theoretical assessment, it is highly probable that this enhanced stability is due to the negative charge of the membrane lipids in Sonazoid®. Negative head groups will lead to electrostatic repulsive intermolecular forces within the stabilizing membrane. Upon stress induced deformation (*e.g.* as during pressure stress) these charges will be brought out of their thermodynamic equilibrium state resulting in a restoring force that (i) will limit further deformation and (ii) will restore the original equilibrium once the stress is relieved. A high density of negatively charged membrane components will therefore inhibit deformation due to stress and increase the stability of the microbubble.

14. For membranes made from predominately neutral lipids, such as with MRX115 and Imagent®, little or no electrostatic repulsion will exist between the head groups. Such membranes will deform more easily and, as shown in the data included herewith, will also disintegrate more easily. This evaluation is corroborated by the fact that Imagent®, which contains 100% neutral lipids, displays the lowest stability of the three agents investigated. The MRX115 membrane contain 82 mole % of a neutral lipid, 10 mole % of a negatively charged lipid and 8 mole % of a positively charged lipid, giving a net negative charge density of 2 mole % (due to the screening of electrostatic interactions by the PEG component the microbubbles display an overall charge of zero). With this small net amount of charge in the lipid membrane MRX115 shows intermediate pressure stability. The Sonazoid® membrane, on the other hand, consists of 100% negatively charged lipids and displays, by far, the best stability of the three.

15. Electrostatic stabilization may also be achieved using *positively* charged lipids. However, as blood proteins are predominately negatively charged, such positively charged

microbubbles would display excessive protein opsonization after administration. This opsonization would lead to activation of the body's immune system and would cause a pronounced adverse, and perhaps toxic effect *in vivo*.

16. In sum, contrary to Mattrey's disclosure, MRX115 and Imagent® are far less suitable as contrast agents because, when used for identification of sentinel lymph nodes, those agents are not stable with regard to pressurization during and after administration. Sonazoid®, in contrast, is far more stable with regard to the same pressurization. Further, unlike positively charged microbubbles, Sonazoid® will not precipitate an immune response when used as contrast agent for the identification of sentinel lymph nodes.

17. I declare further that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge, that willful false statement and the like thereof made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the above-named application or any patent issuing thereon.

Respectfully submitted,

Date: 12 March 2014 By: _____
Per C. Sontum